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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/402,488	02/16/2000	MAURICE MOLONEY	9369-98	6010

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EXAMINER	
STEADMAN, DAVID J	
ART UNIT	PAPER NUMBER
1656	

DATE MAILED: 12/30/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/402,488	MOLONEY ET AL.	
	Examiner	Art Unit	
	David J. Steadman	1656	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *25 October 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,4-10,12-16,18,19 and 48-50* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,4-10,12-16,18,19 and 48-50* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Status of the Application

[1] A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 10/25/2005 has been entered.

[2] Claims 1, 4-10, 12-16, 18-19, and 48-50 are pending in the application.

[3] Applicant's amendment to the claims, filed on 10/25/2005, is acknowledged. This listing of the claims replaces all prior versions and listings of the claims.

[4] Applicant's arguments filed on 9/8/2005 and 10/25/2005 have been fully considered and are deemed to be persuasive to overcome some of the rejections and/or objections previously applied. Rejections and/or objections not reiterated from previous Office actions are hereby withdrawn.

[5] The text of those sections of Title 35 U.S. Code not included in the instant action can be found in a prior Office action.

Claim Objections

[6] Claim 15 is objected to in the recitation of "claim 13 step c)..." It is suggested that, *e.g.,* applicant insert "wherein" after "claim 13" and before "step c)."

Claim Rejections - 35 USC § 112, Second Paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

[7] Claims 1, 4-10, 12-16, 18-19, and 48-50 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

A skilled artisan recognizes that a fusion protein is a fusion of heterologous polypeptides, optionally joined by a linker. This meaning of fusion protein is supported throughout the instant specification. Claim 1 (claim(s) 4-10, 12-16, 18-19, and 48-50 dependent therefrom) is unclear in the recitation of "a chimeric nucleic acid sequence that encodes a fusion protein and that comprises (a) a nucleic acid sequence encoding a pro-peptide...linked in reading frame to (b) a nucleic acid sequence that is heterologous to the pro-peptide." This phrase is unclear because it can be interpreted in the following two different ways: 1) the chimeric nucleic acid sequence encodes a fusion protein *and additionally comprises* a nucleic acid sequence encoding a pro-peptide and a nucleic acid sequence that is heterologous to the pro-peptide or 2) the chimeric nucleic acid encodes a fusion protein, wherein the nucleic acid encoding the fusion protein comprises a nucleic acid sequence encoding a pro-peptide and a nucleic acid sequence that is heterologous to the pro-peptide. It is suggested that applicants clarify the intended meaning of the phrase. In the interest of compact prosecution

and in view of the disclosure of the specification, the examiner has taken the second interpretation and examined the claims accordingly.

Claim Rejections - 35 USC § 112, First Paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

[8] Claims 1, 4-10, 13-16, 19, and 48-50 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method for the preparation of a recombinant polypeptide by transforming an isolated host cell with an expression vector encoding a fusion protein comprising a chymosin pro-peptide as shown in Figure 1 linked to a recombinant protein, culturing the isolated cell *in vitro* to produce the fusion protein and adding a mature form of an autocatalytically maturing aspartic protease that is capable of cleaving the chymosin pro-peptide under *in vitro* conditions to the fusion protein to cleave the pro-peptide from the recombinant protein, optionally wherein the mature form of an autocatalytically maturing aspartic protease that is capable of cleaving the chymosin pro-peptide is added by co-transforming the isolated host cell and culturing the isolated host cell to co-express the fusion protein and the zymogenic form of an autocatalytically maturing aspartic protease that is capable of cleaving the chymosin pro-peptide and activating the autocatalytically maturing aspartic protease by treatment at low pH, does not reasonably provide

enablement for practicing the claimed methods in a host *organism*. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

It is the examiner's position that undue experimentation would be required for a skilled artisan to make and/or use the entire scope of the claimed invention. Factors to be considered in determining whether undue experimentation is required are summarized in *In re Wands* (858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988)) as follows: (A) The breadth of the claims; (B) The nature of the invention; (C) The state of the prior art; (D) The level of one of ordinary skill; (E) The level of predictability in the art; (F) The amount of direction provided by the inventor; (G) The existence of working examples; and (H) The quantity of experimentation needed to make or use the invention based on the content of the disclosure. See MPEP § 2164.01(a). The Factors most relevant to the instant rejection are addressed in detail below.

The breadth of the claims: The claims are so broad as to encompass a method of gene transfer such that the fusion protein is expressed in a host organism and optionally wherein the cleavage of the fusion protein is carried out within the organism or, alternatively, the fusion protein is collected from the host organism and cleaved under *in vitro* conditions. This interpretation is supported by the disclosure which states (in relevant part) "the fusion protein is produced *in vivo* in the host animal" and "[t]he chimeric nucleic acid sequences of the invention may

be directly introduced into cells or tissues *in vivo* using delivery vehicles such as retroviral vectors, adenoviral vectors and DNA virus vectors" (p. 13, line 37 to p. 14, line 2). The enablement provided by the specification is not commensurate in scope with the scope of the claimed invention, which encompasses *in vivo* production of a fusion protein and optionally *in vivo* cleavage of the fusion protein by an aspartic protease. In this case, the specification is enabling only for a method for the preparation of a recombinant polypeptide by transforming an isolated host cell with an expression vector encoding a fusion protein comprising a chymosin pro-peptide as shown in Figure 1 linked to a recombinant protein, culturing the isolated cell *in vitro* to produce the fusion protein and adding a mature form of an autocatalytically maturing aspartic protease that is capable of cleaving the chymosin pro-peptide under *in vitro* conditions to the fusion protein to cleave the pro-peptide from the recombinant protein, optionally wherein the mature form of an autocatalytically maturing aspartic protease that is capable of cleaving the chymosin pro-peptide is added by co-transforming the isolated host cell and culturing the host cell *in vitro* to co-express the fusion protein and the mature form of an autocatalytically maturing aspartic protease that is capable of cleaving the chymosin pro-peptide.

The state of the prior art; The level of one of ordinary skill; The level of predictability in the art: At the time of the invention, the ability to achieve successful gene transfer in a host organism was highly unpredictable as evidenced by the references of Dang et al. (*Clin Cancer Res* 5:471-474) and Fox (*Nat Biotechnol* 21:217). Even after the time of the invention, the art still

recognizes the high level of unpredictability associated with gene transfer as evidenced by Juengst (*BMJ* 326:1410-1411).

The amount of direction provided by the inventor; The existence of working examples: In this case, the specification fails to provide even a single working example of the claimed method being practiced in a host organism. While there is no requirement that the specification disclose a working example of the claimed invention, MPEP 2164.02 makes clear that "[l]ack of a working example, however, is a factor to be considered, especially in a case involving an unpredictable and undeveloped art." As evidenced by the cited references, gene transfer was clearly an unpredictable and underdeveloped art at the time of the invention. Further, the specification fails to provide any specific guidance that would provide the skilled artisan with an expectation of successfully practicing the claimed method in a host organism, *e.g.*, vector used for gene transfer and method of gene transfer.

The quantity of experimentation needed to make or use the invention based on the content of the disclosure: In view of the high level of unpredictability and because the specification fails to provide sufficient guidance for practicing the full scope of the claimed invention, a significant amount of experimentation is required.

Thus, applicant has not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims. The scope of the claims must

bear a reasonable correlation with the scope of enablement (*In re Fisher*, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988). It should be noted that, although the examiner has indicated enabled subject matter, this is no indication that such is supported by the instant specification, claims, and drawings as originally filed.

[9] Claims 12 and 18 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Claims 12 and 18 limit steps c) of the methods of claims 10 and 16, respectively, to being "effected in" the milk, stomach, or gut of an animal. The claims can be broadly interpreted as meaning that the fusion protein is transgenically expressed and cleaved by an autocatalytically maturing aspartic protease in the animal or that the fusion protein is produced outside of the animal and administered to the animal, wherein cleavage occurs in the milk, stomach, or gut of an animal. The analysis of the factors of *In re Wands* is incorporated herein. As noted above, the specification fails to provide a working example of the claimed invention, any specific guidance that would enable a skilled artisan to practice the claimed invention, the prior art does not remedy the deficiencies of the specification, and

there is a high level of unpredictability that the fusion protein would be cleaved in the recited *in vivo* conditions. In view of the lack of guidance and the high level of unpredictability, there is no way to ascertain the amount of experimentation that is required. At least for the reasons stated herein, the specification fails to enable the claimed invention.

[10] RESPONSE TO ARGUMENT: Applicant argues the primary basis for the rejection appears to be the lack of working examples. Applicant argues working examples of *in vivo* cleavage of the pro-peptide are not required and that in view of Example 3 and other disclosure of the specification, a skilled artisan would understand that step c) can be effected under *in vivo* conditions.

Applicant's argument is not found persuasive. The rejection does not rely solely on the lack of working examples in the specification. In this case, the rejection relies on the relevant Factors of *In re Wands* as described and analyzed above. As noted above, the examiner acknowledges that a working example of practicing the claimed method *in vivo* is not required to satisfy the enablement requirement. However, in accordance with MPEP 2164.02, "[l]ack of a working example, however, is a factor to be considered, especially in a case involving an unpredictable and undeveloped art." The specification makes clear that the method is intended to encompass is intended to encompass gene transfer in a host cell of a tissue or living organism (see p. 13, line 37 to p. 14, line 2), which, at the time of the invention (and even now) is considered to be an unpredictable and undeveloped art. While applicant argues Example 3 supports "*in vivo* embodiments," it is noted that this example is conducted *in vitro* with the gut

extract of a red turnip beetle, which a skilled artisan would clearly recognize as not being equivalent to cleavage of a fusion polypeptide that is expressed from a transgenic human. At least for the reasons stated above, the specification fails to enable the full scope of the claims.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

[11] Claim(s) 1, 4, 6-9, 13, 15, and 19 are rejected under 35 U.S.C. 103 (a) as being unpatentable over Ward et al. (US Patent 6,265,204 B1, cited on PTO-892 in the 4/29/2004 Office action) in view of Walsh et al. (*J Biotech* 45:235-241, cited in the 4/29/2004 Office action) and Yonezawa et al. (*Int J Pept Protein Res* 47:56-61, cited in the 3/9/2005 Office action). The claims are drawn to a method for the preparation of a recombinant fusion protein by recombinantly expressing a full-length chymosin pro-peptide fused to a heterologous polypeptide and cleaving the fusion protein with an aspartic protease. It is noted that the claims require that the "heterologous nucleic acid sequence is located immediately downstream of the nucleic acid sequence encoding the chymosin pro-peptide." The C-terminal amino acid of a chymosin pro-peptide is a Phe, and one of ordinary skill in the art would recognize that Met is usually the first amino acid of

a given polypeptide. Although the claims are not so limited, the examiner has directed the rejection to the recited fusion protein having a Phe-Met junction between the chymosin pro-peptide and the heterologous protein.

Ward et al. teaches a nucleic acid encoding a fusion protein, wherein the nucleic acid encodes (from the 5'-end) a signal sequence, a secreted polypeptide, a cleavable linker, wherein the cleavable linker is a chymosin pro-sequence, and two or more desired polypeptides (see particularly columns 7-8 and claim 17). Ward et al. teaches that upon construction of the fusion nucleic acid, it is inserted into an expression vector comprising regulatory sequences that are functional in the host to be transformed, including transcriptional regulatory sequences and transcriptional start and stop sequences (column 11). Ward et al. teach that the fusion protein is produced by transforming an appropriate host cell with the fusion expression vector and culturing the transformant (column 13) followed by cleavage of the fusion protein (column 14).

Ward et al. does not expressly teach the use of an autocatalytically maturing aspartic protease, particularly chymosin, to cleave the chymosin pro-peptide cleavable linker sequence.

At the time of the invention, one of ordinary skill in the art would have recognized that chymosin is an appropriate endoproteinase for cleaving a fusion protein at a Phe-Met junction of a desired protein with an N-terminal chymosin pro-peptide as evidenced by Walsh et al. and Yonezawa et al. Walsh et al. teaches Phe-Met at positions 105 and 106, respectively, of the chymosin substrate κ-casein is a specific chymosin cleavage site. Walsh et al. teaches

expression of a fusion protein having a linker comprising a Phe-Met chymosin cleavage site (p. 237). Walsh teaches "specific" cleavage of the fusion protein at the Phe-Met site at pH 4 and 6.8 by addition of mature chymosin (pp. 236 and 240-241). Yonezawa et al. teaches that mature chymosin cleaves a peptide (substrate II) specifically at a Phe-Met site at the P1-P1' positions (see particularly p. 58, Table 1). While one may argue that the results obtained with short peptides are not representative of a larger polypeptide, it is noted that Walsh et al. teaches that their results are "[i]n agreement with observations of chymosin activity on model peptide substrates" (p. 241, left column, top).

Therefore, it would have been obvious to one of ordinary skill in the art to combine the teachings of Ward et al., Walsh et al., and Yonezawa et al. for practicing the method for fusion protein preparation and cleavage of Ward et al. using mature chymosin as a cleaving agent. One would have been motivated to use chymosin as the fusion protein-cleaving agent in the method of Ward et al. because Walsh et al. teaches that a Phe-Met site is the specific cleavage site of κ-casein and both Walsh et al. and Yonezawa et al. demonstrate that chymosin can cleave a Phe-Met site. One would have a reasonable expectation of success for practicing the method for fusion protein preparation and cleavage of Ward et al. using mature chymosin as the fusion protein-cleaving agent because of the results of Ward et al., Walsh et al., and Yonezawa et al. Therefore, claims 1, 4, 6-9, 13, 15, and 19, drawn to the methods as described above would have been obvious to one of ordinary skill in the art.

[12] RESPONSE TO ARGUMENT: Applicant's arguments filed on 10/25/2005 addressing the instant rejection appear to encompass applicant's arguments presented in the response filed on 9/8/2005 with additional added remarks. As such, the examiner will refer only to the arguments filed on 10/25/2005.

Applicant argues the rejection is improperly based on hindsight reasoning because the prior art does not teach or suggest the use of a mature form of an autocatalytically maturing aspartic protease to cleave a chymosin propeptide from a fusion protein. Applicant argues that prior to the invention, one of ordinary skill in the art would not have recognized that a chymosin pro-peptide could be specifically and accurately cleaved by the addition of a mature form of an autocatalytically maturing aspartic protease. Referring to Examples 1-2, applicant argues the accurate cleavage reported in the specification was surprising and unexpected and satisfies a long-felt need for the claimed method. Applicant argues the "uncertainty" associated with specific cleavage is evidenced by Ward et al. that unwanted amino acids may be present in the protein of interest following cleavage of the fusion protein and that this disclosure teaches away from the claimed invention. Applicant argues that, although not specifically recited, the claims encompass the claims encompass the "concept" of "accurately" cleaving the fusion protein.

Applicant's argument is not found persuasive. Initially, it is noted that the claims are not limited to specific or accurate cleavage of a fusion protein. As such, applicant is arguing a limitation that is not present in the claims. See MPEP 2145 regarding arguing limitations that are not claimed. While applicant alleges

the precise and accurate cleavage of the fusion proteins disclosed in Examples 1-2 is surprising and unexpected, the claims are not so limited to these specific embodiments. In this case, it appears that applicant's current argument contradicts applicant's previous statements of record, which clearly support non-specific cleavage of the fusion protein being encompassed by the claimed method. For example, applicant has previously argued that "the claims do not preclude some non-specific cleavage of the heterologous protein" (response filed on 9/18/2001 at p. 6, bottom). In the same response, applicant states, "[a]pplicant has tested many proteins and has not observed *substantial* non-specific cleavage of any of the proteins" (italics added for emphasis, response filed on 9/18/2001 at p. 7, top), thus suggesting that at least *some* non-specific cleavage has been observed.

Applicant does not dispute that Ward et al. teaches the use of a chymosin pro-peptide as a fusion protein linker in accordance with the claims. What is at issue is whether one of ordinary skill in the art at the time of the invention would have had a reasonable expectation of success that a mature form of an autocatalytically maturing aspartic protease could cleave a chymosin pro-peptide from a fusion protein. Walsh et al. teaches the successful use of a bovine kappa-casein chymosin cleavage site (cleavage between Phe-Met, wherein Phe is at the P1 position and Met is at the P1' position) as a cleavable linker in a fusion protein. The art-recognized pro-peptides of chymosin from mammalian and fungal sources also have a Phe at the P1 position and, if fused to a heterologous protein – most having a Met at the N-terminus – would also produce a Phe-Met

junction. According to applicant, a skilled artisan would not expect an autocatalytically maturing aspartic protease to "precisely" or "accurately" cleave at this site between the chymosin pro-peptide and the heterologous protein. However, the claims require neither *precise* nor *accurate* cleavage at this site, requiring only that "said chymosin pro-peptide is cleaved from said fusion protein to release said recombinant polypeptide." While Ward et al. teaches that "[i]n some embodiments, ... the desired polypeptides contain unwanted amino acids from the amino or carboxy termini" (column 14, lines 33-35), it should be noted that the claimed method also does not preclude unwanted amino acids at the amino or carboxy termini of the recombinant polypeptide. Even assuming *arguendo* a limitation addressing the precision or accuracy of cleavage was present in the claim(s), it is noted that Walsh et al. also teaches that their disclosed fusion protein was cleaved in a "specific" manner by chymosin (p. 240, right column, bottom to 241, left column, top).

MPEP 2143.02 makes clear that absolute predictability is not required, only *some* degree of predictability. In view of the teachings of Walsh et al. and Yonezawa et al., one of ordinary skill in the art at the time of the invention would have had at least *some* degree of predictability that the fusion protein as taught by Ward et al. could be cleaved by an autocatalytically maturing aspartic protease, *e.g.,* chymosin.

[13] Claim(s) 5 is rejected under 35 U.S.C. 103 (a) as being unpatentable over Ward et al. in view of Walsh et al. and Yonezawa et al. as applied to claims 1, 4, 6-9, 13, 15, and 19 above, and further in view of Fine et al. (*Gen Comp*

Endocrinol 89:51-61, cited in the 12/4/2001 Office action). Claim 5 limits the recombinant protein to hirudin or carp growth hormone.

Ward et al., Walsh et al., and Yonezawa et al. disclose the teachings as described above. Additionally, Ward et al. teach that their method can be used to express a fusion protein comprising an epitope for affinity purification of the fusion protein (see column 10, top). None of Ward et al., Walsh et al., or Yonezawa et al. teaches a method for producing a recombinant carp growth hormone.

Fine et al. teaches the recombinant expression of carp growth hormone (cGH) with an N-terminal Met using Escherichia coli as an expression host (p. 52, right column). Fine et al. teaches the cDNA sequence of cGH has been isolated and characterized (page 52, left column, bottom to right column, top).

Therefore, it would have been obvious to one of ordinary skill in the art to combine the teachings of Ward et al., Walsh et al., Yonezawa et al., and Fine et al. for the method of Ward et al. with cGH as the protein of interest produced as a fusion protein with an affinity tag with a chymosin pro-peptide linker. One would have been motivated to practice the method of Ward et al. with cGH as the desired protein in order that: 1) the cGH protein is expressed by a eukaryotic cell and 2) that the cell secretes the protein into the culture medium thereby reducing purification steps and time. One would have been motivated to express cGH with an affinity tage in order to allow affinity purification of the protein. One would have a reasonable expectation of success for the method of Ward et al. with cGH as the protein of interest produced as a fusion protein with an affinity tag with a

chymosin pro-peptide linker because of the results of Ward et al., Walsh et al., Yonezawa et al., and Fine et al. Therefore, claim 5, drawn to the method as described above would have been obvious to one of ordinary skill in the art.

[14] RESPONSE TO ARGUMENT: Applicant argues the teachings of Fine et al. fail to remedy the alleged deficiencies of the cited references. However, this is not found persuasive as the combination of references teaches all limitations of the claims, provides motivation for making the claimed invention, and provides a reasonable expectation of success for practicing the claimed method.

[15] Claim(s) 10, 16, and 48-49 are rejected under 35 U.S.C. 103 (a) as being unpatentable over Ward et al. in view of Walsh et al. and Yonezawa et al. as applied to claims 1, 4, 6-9, 13, 15, and 19 above, and further in view of Ward et al. (*Biotechnol* 8:435-440, referred to herein as "Ward et al. (2)" to prevent confusion with the other Ward et al. reference) and LaVallie (US Patent 5,665,566). Claims 10 and 16 limit step c) to being "effected" under *in vivo* conditions. Claims 48-49 limit step c) to being "effected" by expressing said aspartic protease in said host cell.

Ward et al., Walsh et al., and Yonezawa et al. disclose the teachings as described above. Additionally, Ward et al. teach that their method can be used to express a fusion protein comprising an epitope for affinity purification of the fusion protein (see column 10, top). None of Ward et al., Walsh et al., or Yonezawa et al. teaches a method for producing a recombinant carp growth hormone.

Ward et al. (2) teaches recombinant expression of the zymogenic form of chymosin in a microorganism followed by autoactivation at low pH is well-known in the prior art (p. 435, right column).

LaVallie teaches the recombinant co-expression of enterokinase and a fusion protein comprising an enterokinase cleavage site and combining the expressed enterokinase with the fusion protein by, *e.g.,* cell lysis, for cleavage of the fusion protein (columns 19-20, Example 9).

Therefore, it would have been obvious to one of ordinary skill in the art to combine the teachings of Ward et al., Walsh et al., Yonezawa et al., Ward et al. (2), and LaVallie for the method of Ward et al. modified to co-express a fusion protein and prochymosin in a host cell and lysing the host cell and activating the prochymosin to chymosin by treatment of the lysate about pH 2.0 for cleavage of the fusion protein. One would have been motivated to practice the method of Ward et al. modified to co-express a fusion protein and prochymosin in a host cell and lysing the host cell and activating the prochymosin to chymosin by treatment of the lysate about pH 2.0 for cleavage of the fusion protein in order to produce cleaved fusion protein without need of adding exogenous chymosin. One would have a reasonable expectation of success for the method of Ward et al. modified to co-express the fusion protein and prochymosin in a host cell and lysing the host cell and activating the prochymosin to chymosin by treatment of the lysate about pH 2.0 for cleavage of the fusion protein because of the results of Ward et al., Walsh et al., Yonezawa et al., Ward et al. (2), and LaVallie.

Therefore, claims 10, 16, and 48-49, drawn to the method as described above would have been obvious to one of ordinary skill in the art.

[16] Claim(s) 14 and 50 are rejected under 35 U.S.C. 103 (a) as being unpatentable over Ward et al., Walsh et al., and Yonezawa et al. as applied to claims 1, 4, 6-9, 13, 15, and 19 above and further in view of Dunn et al. ("Aspartic Proteinases", Advances in Experimental Medicine and Biology, Volume 362, Plenum Press, NY, 1995, pp. 1-9, cited in the 4/29/2004 Office action). Claim 14 limits the aspartic protease added in step c) to an aspartic protease that is heterologous to the chymosin pro-peptide and claim 50 limits the aspartic protease to pepsin.

Ward et al., Walsh et al., and Yonezawa et al. disclose the teachings as described above. Yonezawa et al. further teaches substrate II (containing a Phe-Met cleavage site) is "hydrolyzed by pepsin at nearly the same rates for chymosin" (p. 58, Table 1 and p. 61, left column, top). None of Ward et al., Walsh et al., or Yonezawa et al. teaches cleavage of a fusion protein comprising a chymosin pro-peptide using a heterologous aspartic protease, particularly pepsin.

Dunn et al. teaches that a plurality of aspartic proteases, including pepsin have the ability to proteolytically cleave a recognition site having Phe in the P1 position (p. 5).

Therefore, it would have been obvious to one of ordinary skill in the art to combine the teachings of Ward et al., Walsh et al., Yonezawa et al., and Dunn et al. for practicing the method for fusion protein preparation and cleavage of Ward et al. using mature pepsin as a cleaving agent. One would have been motivated

to use pepsin as a cleaving agent to cleave a fusion protein having a Phe-Met junction because of the teachings of Yonezawa et al. that pepsin has a nearly equivalent catalytic specificity for a peptide having a Phe-Met cleavage site. One would have a reasonable expectation of success for for practicing the method for fusion protein preparation and cleavage of Ward et al. using mature pepsin as a cleaving agent because of the results of Ward et al., Walsh et al., Yonezawa et al., and Dunn et al., particularly the teachings of Yonezawa et al. and Dunn et al., which show that pepsin can cleave a peptide having a Phe-Met cleavage site and Walsh et al., which teaches that results obtained for mature chymosin cleavage of a full-length fusion protein are "[i]n agreement with observations of chymosin activity on model peptide substrates" (p. 241, left column, top). Therefore, claims 14 and 50, drawn to the method as described above would have been obvious to one of ordinary skill in the art.

[17] RESPONSE TO ARGUMENT: Applicant argues the combination of prior art fails to teach the claimed invention. Applicant argues that, although mature aspartic proteases have been shown to cleave specific peptides at specific sites, this does not implicate the use of a mature aspartic protease for cleaving the recited fusion protein. Applicant again argues the rejection is based on hindsight reasoning because a skilled artisan would not expect that a fusion protein as recited in the claims could be cleaved by an aspartic protease without also incurring undesired cleavage. Applicant argues that without an assurance of accurate cleavage, there would have been no motivation to use an aspartic protease to cleave the fusion protein.

Applicant's argument is not found persuasive. First, as noted above, the claims are not limited to specific or accurate cleavage of a fusion protein. As such, applicant is arguing a limitation that is not present in the claims. See MPEP 2145 regarding arguing limitations that are not claimed. In this case, it appears that applicant's current argument appears to contradict applicant's previous statements of record, which clearly support non-specific cleavage of the fusion protein being encompassed by the claimed method. For example, applicant's have previously argued that "the claims do not preclude some non-specific cleavage of the heterologous protein" (response filed on 9/18/2001 at p. 6, bottom). In the same response, applicant states, "[a]pplicant has tested many proteins and has not observed *substantial* non-specific cleavage of any of the proteins" (italics added for emphasis, response filed on 9/18/2001 at p. 7, top), thus suggesting that at least *some* non-specific cleavage has been observed.

MPEP 2143.02 makes clear that absolute predictability is not required, only *some* degree of predictability. In view of the teachings as described above, one of ordinary skill in the art at the time of the invention would have had at least *some* degree of predictability that the fusion protein as taught by Ward et al. could be cleaved by an autocatalytically maturing aspartic protease other than chymosin, *e.g.,* pepsin.

Conclusion

[18] Status of the claims:

- Claims 1, 4-10, 12-16, 18-19, and 48-50 are pending.

- Claims 1, 4-10, 12-16, 18-19, and 48-50 are rejected.

- No claim is in condition for allowance.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David J. Steadman whose telephone number is 571-272-0942. The examiner can normally be reached on Mon to Thurs, 6:30 am to 5:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Kathleen Kerr can be reached on 571-272-0931. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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